ARTICLE V.

On the Conversion of Benzoïc into Hippuric Acid. By James C. Booth and Martin H. Boyè. Read May 29, 1843.

THE importance of the application of chemical principles to the various processes in the animal economy, not less than their interesting character in a scientific point of view, having led us to undertake a few experiments on a singular fact observed some years since by Woehler, we have deemed them worthy of being offered to your notice on the present occasion, although time has failed in completing the series of investigations which was contemplated. I therefore propose to offer you a few remarks on the "Conversion of Benzoic into Hippuric acid," which are the results of the conjoint labours of Professor Booth and myself.

Before entering into the details of the present treatise, it may not be out of the way to make a few remarks on some of the most important ingredients of urine, as far as connected with the present subject; urine contains about ninety-three parts of water and seven of solid substances. Of these seven parts of solid substances, a substance called urea forms three parts. About two parts are formed by inorganic substances, such as chlorides of potassium, sodium and ammonium, sulphates of soda and potassa, and the phosphates of lime, magnesia, soda, and ammonia; while the remaining two parts are mostly formed of other organic substances, as extractive matters, mucus of the bladder, some lactic acid and uric acid.

Urea is in every respect the most remarkable substance in the urine, and that which imparts to it its most peculiar characters. It is a white crystallizable substance, and very soluble in water. It seems to stand on the borders between organic and inorganic substances. It may be produced artificially from inorganic substances, and is itself readily converted into carbonate of ammonia by taking up the elements of one atom of water. It combines with some acids, such as the nitric and oxalic acids, and performs in these compounds the part of a base.

Lithic or uric acid forms only a very small part of the urine; being about one-seventieth of the solid substances, and only one-ten thousandth part of the whole urine. It is very insoluble in water, and this is the reason that it for the most part deposits from urine on cooling. By oxidation it yields, among other products, urea. It forms the greater part of urinary concretions.

The urine of herbivorous animals does not contain any uric acid, but another acid, called hippuric acid, from ἵππος, a horse, in whose urine it was first discovered. It only contains seven per cent. of nitrogen, while uric acid contains thirty-three per cent. By oxidation, it yields, among other products, benzoic acid.

The urine of the horse sometimes contains benzoic acid instead of hippuric. Benzoic acid is generally obtained from gum-benzoin by subjecting it to a gentle heat; when it sublimes in crystalline needles. It will be observed that it contains no nitrogen. It belongs to a series of compounds, which in a historical point of view are of interest, as containing the first well established radical in organic chemistry; that is to say, an imaginary body, which, though never isolated by itself, may be carried through a series of combinations with most of the simple elementary bodies.

The question now naturally arises, how is this transformation of benzoic acid into hippuric effected? We have seen that the normal quantity of uric acid is too small to suffice, if its formation depended on this acid, and, besides, their formation seems independent of each other. That the transformation should be effected in the stomach and intestines during the digestion is less probable: it seems more probable to suppose that it takes place during the subsequent circulation of the blood, and that its formation is connected with that of the urea. We know that at least the principal source of urea is the transformation of the tissues in the body, or what Prout calls the destructive assimilation, since its formation continues, when animals are fed exclusively on non-nitrogenized food, and when they are starved; and that it is not secreted by the kidneys, but actually formed in the body, and merely excreted by them, is proved by removing them, when urea will be found in the blood. The same occurs by suppression of urine. I have myself lately had an opportunity to confirm this observation, in examining, at the request of Drs. Pepper and Sargent, the blood of a patient who for some time previous to her death had a complete suppression of the urine. But the question then is,—is it merely a simple chemical reaction between the benzoic acid and the products of the transformations of the tissues, or does it actually affect the chemical changes, by which the latter are effected, so as to cause new chemical products to be their results. Liebig takes this latter view in his Animal Chemistry. We quote his own words:

"Benzoic acid, when administered internally, appears in the form of hippuric acid. Should the observation be confirmed, it will acquire great physiological significance, since it would plainly prove, that the act of the transformation of the tissues in the animal body under the influence of certain matters taken in the food, assumes a new form with respect to the products, which are its results; for hippuric acid contains the elements of one at. of urea, one of lactic acid, and two of benzoic acid."

Liebig then at once proceeds to make an application of this fact to the action of medicinal agents. We are not disposed to dispute his theories on this point, nor deny that such may be the case in active remedies, but we believe that he is too prompt in drawing the conclusion, that because benzoic acid is converted into hippuric by entering into the system, it should, therefore, necessarily affect the usual chemical changes in the body. We are disposed to consider it as a mere chemical reaction between the benzoic acid and the substances of the blood, either those which are used for the formation of the tissues, or, more probably, those which result from their dissolution. But if this be correct, it fol-

lows, that we may be able to perform the same transformation out of the body. It was with a view of determining this point that our experiments were undertaken, and we still look for success, although they have not hitherto afforded a satisfactory result.

Woehler was the first to observe that benzoic acid, when administered internally to a dog, and again separated from the urine, reappeared as prismatic crystals, unlike benzoic acid; and he conjectured correctly, from their resemblance to hippuric acid, that the former by passing through the system was converted into the latter. He also suggested, that this might be the source of the hippuric acid in the urine of herbivorous animals, since the benzoic acid might be supposed to occur in their food, and by the digestion be converted into hippuric. Ure subsequently stated, that he had found hippuric acid in the urine of a patient who had taken benzoic acid; he also supposed that the uric acid had been employed in this conversion, and recommended it as a remedy for calculous concretions of uric or lithic acid. This induced Keller, at the instigation of Woehler, to make some experiments on this point, which he published in Liebig's Annals of Chemistry and Pharmacy. He took, in the evening before bed-time, two grammes or about thirty-one grains of benzoic acid, and found the next morning, on examining the urine, that by the addition of chlorohydric acid it deposited long prismatic crystals, which it continued to do as long as he continued the use of benzoic acid. By re-dissolving these crystals in hot water and treating them with animal charcoal, he obtained them perfectly colourless. The crystals thus obtained behaved in all respects like pure hippuric acid. They fused easily, and by a stronger heat they charred with a smell of oil of bitter almonds and sublimation of benzoic acid. He also determined the quantity of carbon, which he found to agree with that of hippuric acid, namely, 60.7 per cent., while benzoic acid contains 69.1 per cent. of carbon.

Keller furthermore ascertained that the urine from which hippuric acid had been separated, deposited a large amount of nitrate of urea, on the addition of nitric acid. It had also previously deposited uric acid. It therefore contained these two usual ingredients of urine. He also inferred, that since the urine could be inspissated without depositing hippuric acid before the addition of chlorohydric acid, it was evident that the hippuric acid existed in combination with a base.

As hippuric acid may be said, to a certain extent, to replace uric acid in the herbivorous mammalia, this probably led Ure to the supposition that the uric acid was employed in the conversion of the benzoic acid; but Keller's experiment showed that the urine deposited uric acid, and that therefore this latter assertion could not be correct.

We have repeated Keller's experiments, and with the same results. We have in general found that after the use of benzoic acid the urine makes a copious deposite of uric acid, both in the red or dark-coloured variety and in that of a clay-coloured or muddy appearance.

In one experiment the urine was examined in the morning when no benzoic acid had been taken. It deposited no sediment on cooling, and when tested for uric acid by the addition of nitric acid, the latter effected after twenty-four hours a deposite of only a few isolated colourless crystals scattered over the inside of the vessel. The same day two grammes of benzoic acid were taken immediately after dinner. Twenty minutes afterwards the urine was voided, evaporated to one-fourth its volume, and chlorohydric acid

added. After the lapse of twenty-four hours it had deposited a precipitate of a granular appearance and of a very dark-red nearly black colour, which dissolved with effervescence in nitric acid, and on evaporation yielded the beautiful purple colour of purpuric acid. It therefore consisted of uric acid. Twenty minutes after the first, another portion was obtained, which, treated in the same way, yielded a precipitate in all respects similar to the above. Its quantity also had not diminished; but besides this precipitate there appeared a moderate quantity of needle-shaped crystals of hippuric acid.

These experiments evidently show that the formation of hippuric acid does not diminish the quantity of the uric acid, and that the formation of the former seems entirely independent and disconnected with that of the latter. The urine voided in the morning had a sp. gr. of 1.0112. That voided three or four hours after dinner, and therefore containing most of the hippuric acid, had a sp. gr. of 1.024. Its colour was but slightly more yellow than the former.

Distinction is generally made between the urine secreted after fluids have been taken, especially on an empty stomach, and that secreted subsequently to the digestion of food. The former is much more limpid and tasteless, and contains much less of the solid ingredients. It has been found, that if any substance be taken under the first circumstances, dissolved in the water, it passes much more rapidly into the urine, where some substances have been detected in the short time of from two to ten minutes after their introduction into the stomach; while, when taken with the food, they generally require a much longer time for their reappearance in the urine. In regard to the time which elapses before the benzoic acid reappears as hippuric, it will be seen that in the above experiments it had not yet appeared there in twenty minutes, and that forty minutes after being taken its appearance had but fairly commenced. In another experiment, where the benzoic acid was taken immediately before dinner, a portion of urine, obtained immediately after the meal, and about thirty minutes after the introduction of the acid yielded, when treated as above, an abundant crop of crystals. It was interesting to ascertain how long the hippuric acid would continue to appear in the urine from the time the benzoic acid was taken. In an experiment, where two grammes were taken before and two grammes more immediately after dinner, the urine secreted (from seven to eleven o'clock in the evening or) from four to eight hours after its introduction, contained an abundance of hippuric acid, while none could be detected in the urine voided after that time the next morning.

The quantity of hippuric acid obtained exceeds that of the benzoic acid taken; six grammes taken, two grammes after breakfast, two before and two after dinner yielded in two experiments about eight grammes of crude hippuric acid, or about one-third more than its own weight.

It was a matter of considerable interest to ascertain with what base the hippuric acid occurred combined in the urine, whereby it was held in solution till liberated by an acid. The bases occurring in the urine are potassa, soda, lime, magnesia, and ammonia. Besides these, urea seems in some instances to have a basic nature: thus, in the compounds which it forms with nitric and oxalic acids it evidently acts the part of a base. Cap and Henry have asserted that a portion of it occurs combined with lactic acid in human urine and with hippuric acid in urine of the horse, and they prescribed a method of forming these two compounds by double decomposition of lactate or hippurate of lime and oxalate of

urea, and they also described the methods by which they had extracted the lactate of urea from human urine and the hippurate of urea from the urine of the horse. The former was repeated by Lecanu, who could obtain no lactate of urea. Cap and Henry replied, and gave a new method of obtaining it in crystals by shaking the inspissated urine with a mixture of one volume of alcohol and two volumes of ether, and evaporating the solution by heat over sulphuric acid in a closed vessel. Very recently Pelouze has shown, that neither lactate nor hippurate of urea can be obtained by double decomposition, or in any other way, and that they therefore do not exist, and that Cap and Henry had nothing but urea, which had imbibed the acids.

If hippurate of urea therefore existed, it would not be improbable that this acid might occur combined with urea in the urine, and in our experiments particular attention was paid to this point. The urine was evaporated, filtered, and further evaporated in a water-bath to a thick syrup, and then left at rest for several weeks, in the hope that the combination of hippuric acid would crystallize. A quantity of crystals appeared, which, being well drained from the mother-liquid, were found to contain no hippuric acid. They consisted chiefly of chlorides of ammonium and sodium, with the phosphates of the same bases. The mother-liquid, on the contrary, formed nearly a solid mass when tested for hippuric acid by the addition of chlorohydric acid. By adding more water, and straining the mother-liquid from the crystals, subsequent addition of nitric acid caused an abundant precipitate of nitrate of urea. The main mass of the syrup was now shaken for several days in a closed bottle with a mixture of one volume of alcohol and two volumes of ether, which were decanted and evaporated by heat over sulphuric acid. While concentrating, it yielded prismatic crystals, and finally the whole was converted into a mass of crystals with but little mother-syrup. The crystals were carefully dried between blotting paper. They were, therefore, obtained according to Cap and Henry's directions for extracting the lactate of urea; but they proved to be pure urea, so that unless the lactic acid has been employed in the formation of hippuric acid, lactate of urea is not obtained in this way, and at all events it shows that urea is also dissolved by this process. The same process of extraction was repeated with a fresh portion of the same mixture, and with the same results; but the evaporation was not carried so far, in order to be able to examine the mother-liquid. We obtained a crop of crystals of pure urea, containing no hippuric acid, while the mother-liquid had a bitterish taste but did not yield hippuric acid by the addition of chlorohydric acid. It was therefore evident that the above mixture extracted pure urea, while it left behind the combination of hippuric acid. We next employed a mixture of equal volumes of alcohol and ether, which dissolved a much larger quantity from the original syrup, but on evaporation yielded only a slight crop of crystals. The greater part of it remained as a thick syrup, yielding an abundance of crystals of hippuric acid by the addition of chlorohydric acid, but also a large amount of nitrate of urea by the subsequent addition of nitric acid. It was therefore evident that the compound of hippuric acid was also extracted by the last treatment, and it merely remained to find the base. A few drops of the syrups charred and incinerated, left no residue, proving the absence of all the fixed bases of the urine. The hippuric acid could therefore only be combined with urea or ammonia, to ascertain which chlorohydric acid was added to a portion of it, and after the separation of the hippuric acid, chloride of platinum added, which caused a very copious

precipitate of chloroplatinate of ammonium. The addition of nitrate of silver and nitric acid to the liquid proved it to contain a certain amount of chlorine as chloride of ammonium, but much less than would be able to yield a precipitate so copious as the above. We, therefore, continued the extraction by fresh portions of equal volumes of alcohol and ether, and thus succeeded in obtaining a solution that yielded by evaporation a viscid mass, which, by addition of chlorohydric acid, gave an abundance of hippuric acid, and by subsequent addition of chloride of platinum a corresponding copious precipitate of chloroplatinate of ammonium; but on the addition of nitric acid it yielded no nitrate of urea nor any trace of this substance by evaporation, but only copious crystals of chloride of ammonium. It was thus made evident that urea is not combined with the hippuric acid, but that the base which retains it in solution and with which it is combined is ammonia. It also confirms the assertion of Pelouze that hippurate of urea does not exist.

The results of the foregoing experiments may be summed up thus:

1. The formation of uric acid in healthy urine is not affected either in regard to its quantity nor to its external properties in general by the introduction and transformation of benzoic acid into hippuric acid in the system.

2. The time required for the benzoic acid to pass through the system, and reappear as hippuric in the urine, is from twenty to forty minutes after its introduction with food into the stomach. Its occurrence continues for four or eight hours, but then ceases.

3. The quantity of hippuric acid obtained from the urine is greater than that of the benzoic acid taken. In round numbers it may be stated to be one-third more.

4. Urea is not in combination with the hippuric acid in the urine.

5. The base with which the hippuric acid is combined, and which keeps it in solution, is ammonia.